Back to Basics - 10

Resolution in Microscopy and Photography

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This back to basics article answers two questions:

1. Why do you always hear 'All you need for photography through the microscope is a 3 megapixel camera'?
2. Why are my photos not as sharp as I'd like them to be, especially at higher magnifications? Both questions are primarily an issue of Resolution.

I will discuss the practicalities for us as microscopists and give references to the theory, if you want to pursue this further.

Resolution

"The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities" [1].

Try this practical experiment. Look at the eight columns of dots below in Figure 1.

If we can see an eighth column, we have a damn good printer! In which column can you still see discrete small dots? That column indicates the finest resolution for your eyes. Our newspapers, magazines, television, computer and smartphone screens all exploit our limits of resolution. They use very closely spaced spots or lines that we cannot resolve by eye (Figure 2), and so merge into a picture for us.

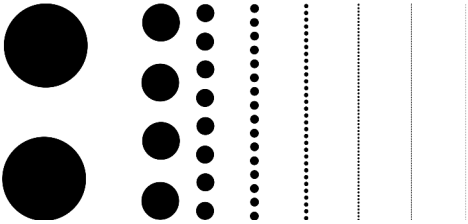

Fig 1: Evenly spaced dots, decreasing by half in each column, showing how, beyond a certain size limit, it becomes difficult to separate the dots and you see a line.

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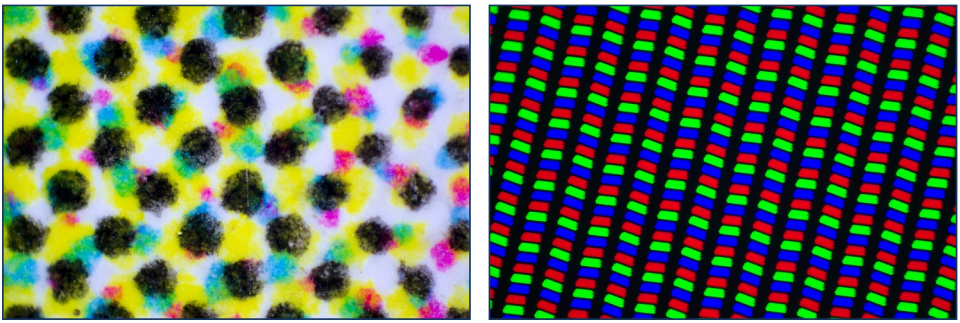

Figure 2. Printed magazine image and smartphone screen at 100x magnification to show that they use small dots or shapes to create an image. The dot spacing is beyond the resolution of your eyes.

Resolution is a feature of the viewing system (our eyes, the objective used). There is an ultimate limit with conventional light microscopy, we cannot theoretically separate dots closer than 0.2 µm (0.0002 mm), due to the nature of light [2]. This gives a maximum real magnification of 1,500x. In practice, 0.25 µm is our limit.

Objectives and Resolution

The maximum resolution that can be achieved by your microscope is determined by the objective you are using. This is indicated on the objective by the NA, its Numerical Aperture [3]. Figure 3 shows the NA plotted against resolution for white light. The curve and the table show that initially, small changes in NA increase resolution quite dramatically. A x4 objective with NA 0.1 resolves points separated by 3 µm; the usual x40 objective, NA 0.65, resolves objects about 0.5 µm apart. The change from a x40 objective to a x100 oil immersion objective, NA 1.3, takes you down a little bit more to a resolution of 0.25 µm.

Note: You must set up your lighting and condenser properly for each objective. Your condenser MUST have an NA that is equal to or greater than that of the objective. A x100 objective, NA 1.3, will only resolve to 0.35 µm with a condenser of NA 0.95.

Impact of light colour for resolution and vision

Colour, as we perceive it, is defined by the wavelength of light. The reality for a microscopists is that you get a better resolution using shorter wavelength blue light than longer wavelength red light. This is shown in Figure 4. The improvement in ability to see detail is about 31%, or nearly a third more.

So why do microscopists use green filters for best resolution work instead of blue ones? It is a function of our biology. Our eyes have specific cones that sense either mainly red, green or blue light. The colour sensitivities of the different cones overlap. For human eyes, green light gives us maximum sensitivity, because it stimulates ALL the rods and cones in our eyes, as well as having the benefit of improved resolution at very high magnifications [4].

However, for most of our day to day microscopy, it is perfectly fine to use white light with all objectives, as the benefits of colour vision outweigh the marginal improvement in resolution by submitting to a green world.

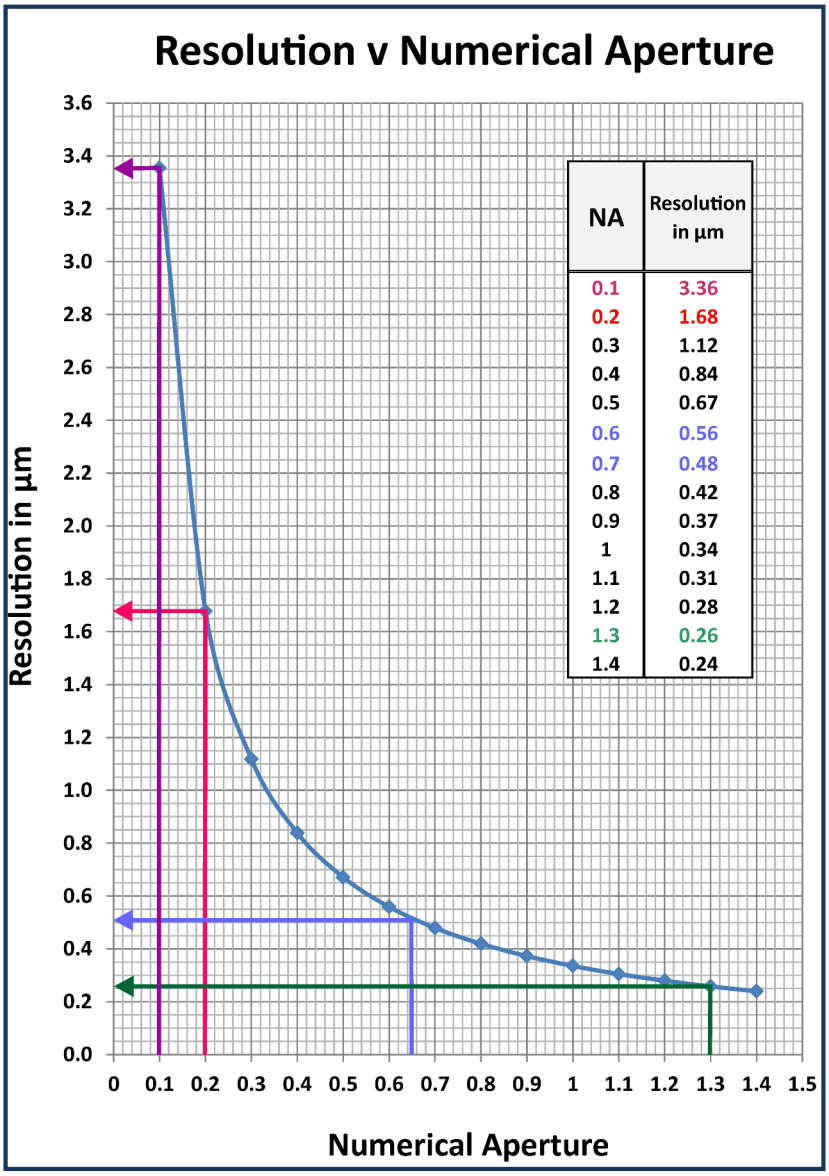

Fig. 3: A microscopist's guide to finding the resolution of an objective from its Numerical Aperture when using white light (average wavelength 0.550 µm). You can use both the graph and the inset chart.
Examples given: Purple – x4 objective, NA 0.1, resolution 3.36 µm Red – x10 objective, NA 0.2, resolution 1.68 µm; Blue – x40 objective, NA 0.65, resolution 0.5 µm; Green - x100 Oil Immersion objective, NA 1.3, resolution 0.26 µm.

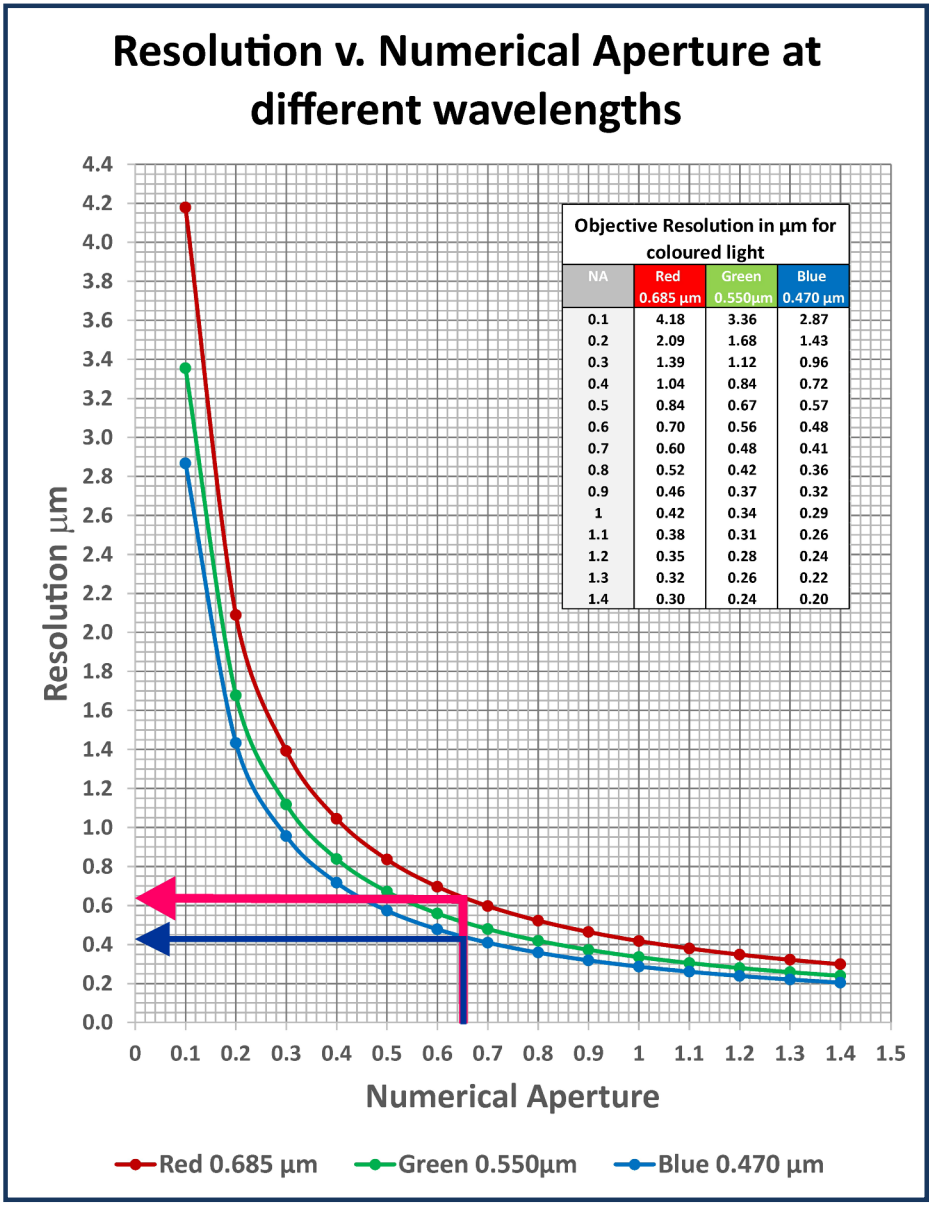

Fig 4. The relationship between objective numerical aperture NA and resolution with red, green and blue light. Example: The resolution of a x40 objective, NA 0.65; with red light = 0.64 µm; with blue light = 0.44 µm.

Field of View and Resolution

Having determined a current objective's resolution, how many resolvable units can we see in our field of view down the microscope? I set up my microscope – camera and took photographs of a 1 mm graduated micrometer scale, which had 100 µm and 10 µm divisions, with all my frequently used objectives (example in Figure 5).

I was able to measure my width and height of **field of view** for each objective in µm.

If you do not have a micrometer scale, do not despair. Photograph two 1 mm bars on a plastic ruler with a x10 objective. Then, photograph a pale coloured picture area from some junk mail leaflet, also with the x10 objective. You can then relate your 'spot scale' to your 'mm ruler scale' and work out how far the spots are apart. Using your 'spot scale', you can measure the size of your field of view with any objective (Figure 6).

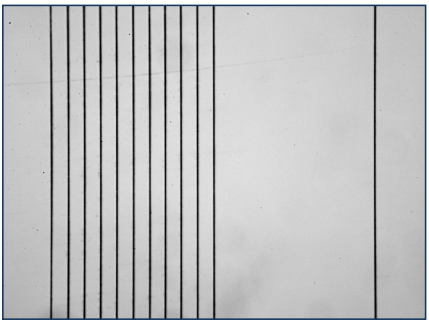

Fig 5. Micrometer scale showing 200 µm, of which 100 µm divided into 10 µm lines. Photographed using a x40 objective on my Reichert Zetopan. Using the scale, I measured the image width as 280 µm and image height as 180 µm.

Calculating the minimum camera resolution needed

Knowing our objective's numerical aperture and having measured our field of view with each objective, we can calculate the absolute minimum resolution of our camera that we require at each magnification. Figure 7 (overleaf) gives a simple five step method you can follow.

Why do we need twice as many pixels relative to resolvable units (both horizontally and vertically) to capture all the information in a particular field of view? Figure 8 demonstrates the effect. We need at least twice 2 x 2 pixels per resolved dot so that the camera can record the separation between the dots.

The third row of figure 8 shows what you would actually see on your photo (printed or on screen), if you were

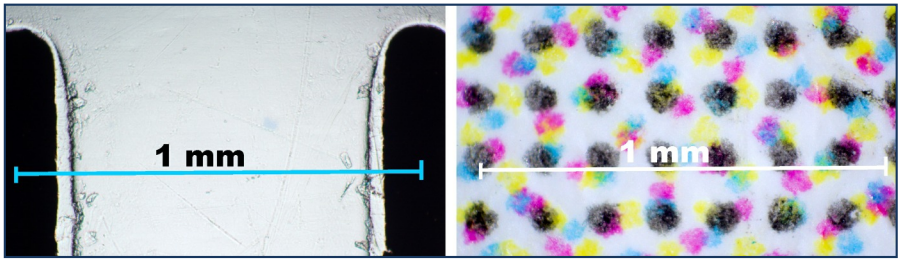

Figure 6. Making your own micrometer scale for photography. Left: A plastic ruler showing two 1mm bars with a x10 objective. Right: Light coloured area on junk mail leaflet, with same x10 objective and photo set up. At about 7.8 spots per mm, the distance between the centres of two spots is about 128 µm.

STEP 1.
Determine the **Resolution** of your objectives from their **Numerical Aperture** and the table or graph in Figure 3.

Objective		Resolution
Magnification	NA	(µm)
4x	0.1	3.36
10x	0.2	1.68
25x	0.45	0.75
40x	0.65	0.52
40x	0.95	0.35
100x	1.25	0.27

STEP 2.
Measure your field of view (FOV) using a micrometer slide or your 'spot scale' for the width and height of standard photos taken with each objective

Camera FOV	
width (µm)	height (µm)
2900	1933
1160	773
515	343
280	186
280	186
115	77

STEP 3.
Calculate how many points you can resolve across the width and the height of your photos by dividing the FOV by the Resolution.

Resolvable units	
width (µm)	height (µm)
864	576
692	461
691	460
542	360
793	527
428	287

STEP 4.
Calculate the minimum number of pixels your camera must record for the width and the height of your photo by multiplying the resolvable units by 2 for each objective. The minimum number of Megapixels (millions of pixels) required by your camera is the result of multiplying pixels wide by pixels high and dividing by 1 million.

Mimimum Camera resolution required		
width (px)	height (px)	total Mpx
1729	1152	2.0
1383	922	1.3
1382	920	1.3
1085	721	0.8
1586	1053	1.7
857	574	0.5

Figure 7. The five steps to calculate to obtain the minimum number of pixels required by a camera to capture all the information in a photograph. The example given is using my microscope objectives and my camera set up. Note the difference in resolution between the two x40 objectives!

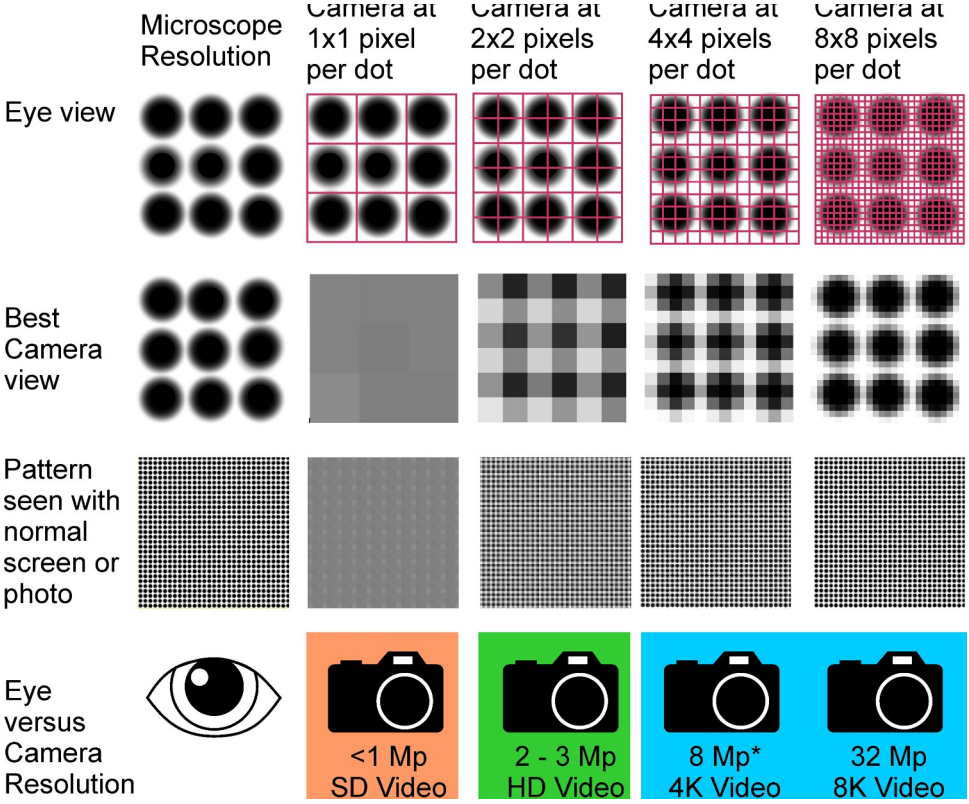
Microscope Resolution
Camera at 1x1 pixel per dot
Camera at 2x2 pixels per dot
Camera at 4x4 pixels per dot
Camera at 8x8 pixels per dot
Eye view
Best Camera view
Pattern seen with normal screen or photo
Eye versus Camera Resolution
<1 Mp SD Video
2 - 3 Mp HD Video
8 Mp* 4K Video
32 Mp 8K Video

Figure 8. **Top row:** What you see by eye and how increasing grids of pixels cover the nine dots shown. **2nd row:** Expected pattern, followed by what the camera actually records with increasing grid of pixels. **3rd row:** Actual pattern when looking at an array of dots under the microscope that can just be resolved, completely filling your field of view. 1 x1 pixels per dot gives no resolution (a blur). 2x2 pixels gives resolution. More than 2x2 pixels per dot makes a marginal difference. **4th row:** Eye and cameras with different resolutions in megapixels (Mp). Orange: Camera resolution less than microscope resolution; Green: Camera exceeds microscope resolution; Blue: cameras vastly exceed microscope resolution. Most digital cameras at time of publication are 8MP or more. *(35mm film cameras have a minimum resolution of 8 Mp or more)

looking down the microscope at a grid of just resolvable dots that filled your field of view. You can see that resolution only occurs if you have **at least** 2 x 2 pixels for each resolvable dot. With only 1 x 1 pixel per dot you just have a blur. Increasing the camera resolution further, to a 4 x 4 or 8 x 8 grid per resolvable dot, the final picture is not much different, you may just have a little more contrast in your photo. Finally, I've given you the equivalent digital camera/video camera and its resolution in the fourth row of Figure 8. Any camera with 2 Mp (megapixels) or more will capture all the detail in photos through all my objectives (green Camera). Most modern cameras are now at least 8 Mp or more, even in smartphones! They will easily capture all your resolvable detail with your microscope and objectives. Because their picture resolution vastly exceeds the resolution of the microscope,

I have coded them blue (they use up more of your computer memory space for little gain). Note, 35 mm film cameras are equivalent to 8 Mp digital cameras or higher.

What the eye and the camera see through the microscope

When looking down my microscope by eye, things always look pretty sharp, until I'm trying to see bacteria or diatom spots at the highest magnifications. Yet when I take photos, they seem to lack the same impact and resolution.

One part of this is my eyes' and brain's fault (and yes, yours too). We see a well illuminated disc framed by a dark background, central in our vision. Our brain boosts the contrast and also emphasises the edges to objects.

Modern digital cameras and old film cameras vastly exceed the minimum resolution required to photograph objects through the microscope (about 2 megapixels). Enlarged micrographs show empty magnification and simply look blurry, unless you artificially sharpen them.

One solution is to create tiled images, where several photos are taken and combined side to side or in a grid. These images have more information in the form of resolvable points and look sharper, when displayed or printed at the same size as a single photo.

Conclusion

This article describes resolution in practical terms for the general microscopist. I show you how you can calculate the resolution and field of view for your objectives. We see how the objective resolution relates to the minimum resolution a camera needs to take a good photo. Any camera with 3 megapixels or greater is more than adequate for photography through the microscope. Tiling several micrographs of a subject is one solution to getting sharper looking photos.

References

1. https://www.microscopyu.com/microscopy-basics/resolution
2. https://en.wikipedia.org/wiki/Microscopy
3. http://micro.magnet.fsu.edu/primer/anatomy/numaperture.html
4. https://en.wikipedia.org/wiki/Color_vision

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